Remarks

Claims 1, 5-9, 14, 20, 22-25, 27, 29, 35-39, 41, 42, 44-49, 51-53, 55-59, and 69-84 are pending. Claims 2-4, 10-13, 15-19, 21, 26, 28, 30-34, 40, 43, 50, 54, and 60-68 have been cancelled. Claims 71-84 are newly added. Claims 1, 24 and 25 have been amended to more clearly claim what Applicants believe to be their invention. Claims 24 and 25 were amended to recite "process" rather than "method" in order to make these claims consistent with base claim 1.

Claim 1 has been amended to recite a process for selectively amplifying nucleic acid sequences comprising contacting multiple single stranded non-circular "degenerate" oligonucleotide primers (P1) rather than "random" oligonucleotide primers (P1). Support for the amendment can be found at least on page 6, lines 11-22 where use of degenerate primers in the claimed methods is described. Claim 1 has been amended to delete "at least one such dNTP renders the TS-DNA resistant to nuclease activity following incorporation thereinto." Support for this amendment can be found at least in original claim 1. Claim 1 has also been amended to recite that "the TS-DNA is labeled during or following synthesis." Support for this amendment and for new claims 71-84 can be found at least from page 18, line 18, to page 20, line 8, and in the Examples (pages 28-42) which involve labeling of TS-DNA.

Claim Interpretation

The Office Action on page 3, line 21 – page 4, line 1 alleges that Applicants have defined the term "random primers" to only mean that random oligonucleotide primers (P1) have nucleotide sequences unrelated to the nucleotide sequences of the amplification target circle (ATC) that acts as a template for amplification. The Office Action further alleges that the result of such a random relationship is that the locations on the ATC at which said random primers hybridize will also be random. (See Office Action page 4, lines 1-2). The Office Action fails to appreciate that what makes an oligonucleotide primer satisfy the requirements of the claimed random oligonucleotides extends beyond the mere relationship with the ATC. Random oligonucleotides are defined on page 6, lines 23-26 where the specification provides "Random refers to an oligonucleotide in which each of the nucleotide positions is occupied by a base selected

at random from among a complete set of possibilities, but commonly limited to the four nucleosides, dAMP, dCMP, dGMP, or dTMP." As such, the oligonucleotide is itself a random compilation of nucleosides, in no particular order nor no particular nucleoside is selected for each position on the oligonucleotide. As such, Applicants submit the Office Actions definition of "random oligonucleotide" is lacking and improper.

Rejections Under 35 USC § 103

1. Claims 1, 5-9, 14, 20, 22-25, 27, 29, 31, 33, 35-38, 39, 41, 44-49, 51-53, 55-58, 69 and 70 were rejected under 35 U.S.C. § 103(a) as being unpatentable over Lizardi (U.S. Patent No. 5,854,033), Landers et al. (U.S. Patent No. 6,703,228), Navarro et al. (J. Virol. Meth., vol. 56, pp59-66, 1996) and Eckstein et al. (Trends in Bioch. Sci., vol. 14(3), pp. 97-100, 1989). Applicant respectfully traverses this rejection to the extent it applies to the claims as amended.

Lizardi discloses a method of rolling circle amplification (RCA) involving replication of single-stranded DNA molecules (see column 19, lines 21-23). Lizardi discloses use of a rolling circle replication primer of defined sequence that hybridizes to an amplification target circle (ATC) followed by rolling circle replication of the ATC primed by the rolling circle replication primer to produce a tandem sequence DNA (see column 19, lines 20-31). Lizardi fails to disclose or suggest explicitly the use of primers having degenerate sequence, fails to disclose or suggest directly hybridization of a plurality of primers to each amplification target circle and fails to disclose or suggest directly formation of multiple tandem sequence DNA products by extension of multiple primers.

Landers et al. discloses PCR methods of genotyping including degenerate oligonucleotide primed-PCR (DOP-PCR) and arbitrarily primed PCR (AP-PCR) (see column 15, line12 – column 16, line 26 and column 17, lines 28-29). AP-PCR utilizes short oligonucleotides with defined sequences that are arbitrarily selected as PCR primers to amplify a discrete subset of portions of a high complexity genome (see column 17, lines 28-33 of Landers et al.).

Navarro et al. discloses PCR-based methods for amplifying sequence present in small circular RNAs.

Eckstein et al. discloses nuclease resistant primers.

Claims 1, 5-9, 14, 20, 22-25, 27, 29, 35-39, 41, 44-49, 51-53, 55-58, 69 and 70 are drawn to a process for selectively amplifying nucleic acid sequences. The process involves contacting multiple single stranded non-circular degenerate oligonucleotide primers (P1) and one or more single stranded amplification target circles, where each ATC hybridizes to a plurality of the P1 primers, under conditions that promote rolling circle replication of the amplification target circle by extension of the P1 primers to form multiple tandem sequence DNA (TS-DNA) products. Thus the claims require amplification of a nucleic acid sequence that involves the use of single stranded amplification target circles, hybridization of a plurality of P1 primers of degenerate sequence to each ATC under conditions that promote rolling circle replication of the ATC by extension of the P1 primers to form multiple TS-DNA products by extension of the P1 primers. As such, the amplification of the nucleic acid sequence is a product of rolling circle amplification primed at different sites on the template ATC and results in multiple TS-DNA products by extension of the P1 primers.

In rejecting a claim under 35 U.S.C. § 103, the Examiner must establish a *prima facie* case that: (i) the prior art suggests the claimed invention; and (ii) the prior art indicates that the invention would have a reasonable likelihood of success. *See In re Dow Chemical Company,* 837 F.2d 469, 5 U.S.P.Q.2d 1529 (Fed. Cir. 1988); *In re Geiger*, 815 F.2d 686, 2 U.S.P.Q.2d 1276 (Fed. Cir. 1987). In order for a reference to be effective prior art under 35 U.S.C. § 103, it must provide a motivation whereby one of ordinary skill in the art would be led to do that which the applicant has done. *See Stratoflex, Inc. v. Aeroquip Corp.*, 713 F.2d 1530, 1535, 218 USPQ 871, 876 (Fed. Cir. 1983). The Patent Office has the burden under § 103 to establish a *prima facie* case of obviousness, which can be satisfied only by showing some objective teaching in the prior art would lead one to combine the relevant teachings of the references. *See In re Fine*, 837 F.2d 1071, 1074 (Fed. Cir. 1988).

The present claims require the use of degenerate primers for priming rolling circle replication of single stranded amplification target circles. Degenerate primers are defined in the current specification on page 6, lines 11-22 where the specification provides "Degenerate refers

to an oligonucleotide in which one or more of the nucleotide positions is occupied by more than one base, i.e., a mixture of oligonucleotides of defined length in which one or more positions of an individual member of the mixture is occupied by a base selected at random from among more than one possibilities for that position."

The Office Action alleges (page 9, lines 16-20) that it would have been *prima facie* obvious to one of ordinary skill in the art to have included random PCR primers of arbitrary, defined sequences in the method of Lizardi. The Office Action argues that those of skill in the art would have been motivated to do so because Landers et al. allegedly provides that random primers allow amplification of unknown DNA sequences. Applicants disagree. Applicants first note that the claims have been amended to clam "degenerate primers" rather than random primers. As such, Applicants submit that Landers et al. neither discloses nor suggests degenerate primers as claimed.

The Office Action alleges, in part, at page 4, lines 17-23, that Lizardi teaches a method of amplification comprising contacting multiple single stranded non-circular random oligonucleotide primers (P1), one or more single stranded smplification target circles (ATCs) under conditions where each ATC hybridizes to a plurality of said P1 primer, wherein said conditions promote rolling circle replication of said ATC by extension of the P1 primers to form multiple tandem sequence DNA (TS-DNA) products. The Office Action admits that Lizardi et al. does not teach random primers (See Office Action page 7, line 20). Applicants further submit that Lizardi et al. does not teach degenerate primers.

In support of its interpretation of Lizardi, the Office Action (page 5 and page 7) cites column 25, lines 36-57 as well as column 28, lines 8-18 of Lizardi et al., which describe strand displacement cascade amplification (SDCA). The Office Action alleges that SDCA teaches the use of multiple primers hybridizing to and priming rolling circle replication of an ATC. This is not the case. SDCA begins with rolling circle replication of an ATC primed by a rolling circle replication primer to form TS-DNA. Secondary strand displacement is accomplished by hybridizing secondary DNA strand displacement primers to TS-DNA (See Lizardi et al. column 25, lines 24-28). Secondary DNA strand displacement primers hybridize to, and prime

replication of, TS-DNA to form what is termed TS-DNA-2. The secondary DNA strand displacement primers have sequence matching part of the OCP or ATC used to generate TS-DNA (See column 12, lines 21-29), therefore, by definition, they do not bind to the ATC (See Lizardi et al. column 25, lines 43-45). Tertiary DNA strand displacement primers then hybridize to, and prime replication of the TS-DNA-2 generated from the replication of the initial TS-DNA, primed by the secondary DNA strand displacement primers to form TS-DNA-3 (See Lizardi et al., column 27, lines 2-4).

While it is true that the methods of Lizardi generically encompass the use of multiple primers and that in some embodiments of the methods of Lizardi the use of rolling circle replication primers and tertiary DNA strand displacement primers together might result more than one primer hybridizing to a single amplification target circle, Lizardi does not specifically disclose and certainly does not suggest the use of multiple rolling circle replication primers nor formation of multiple tandem sequence DNA products from multiple primings of a single amplification target circle. It is clear that the mere fact that certain subject matter is disclosed within a broader generic disclosure does not make obvious the specific subject matter not specifically disclosed. In re Baird, 16 F.3d 380, 382, 29 USPQ2d 1550 (Fed. Cir. 1994). In the case of an obviousness rejection, an inherent but obscure feature of a reference cannot provide the suggestion or motivation to use such a feature in combination with other references. The cited references must suggest what is claimed, not merely inherently disclose disparate elements of the claimed invention, which is all that the present rejection provides. None of Landers et al., Navarro et al. and Eckstein et al. provide any disclosure or suggestion regarding rolling circle replication and thus do not provide any suggestion to focus the method of Lizardi on the claimed use of multiple primers and multiple primings.

The Office Action also alleges at page 8, lines 1-3, that Landers et al. teaches the use of "multiple arbitrary (= random) primers" to amplify double-stranded DNA circles (YACs). This is not the case. Applicants first submit that Landers et al. does not disclose random primers or degenerate primers as claimed. Because the current claims are drawn to the use of degenerate primers, Applicants will direct their arguments to the use of degenerate primers. However,

Applicants maintain that Landers et al. fails to disclose random primers as claimed. As described above, degenerate primers are defined as an oligonucleotide in which one or more of the nucleotide positions is occupied by more than one base, i.e., a mixture of oligonucleotides of defined length in which one or more positions of an individual member of the mixture is occupied by a base selected at random from among more than one possibilities for that position. In other words, each nucleotide position is occupied, at random, with a base. The result is that each primer is random to one another and each primer can be generated without knowledge of the target sequence to which it will bind. The Office Action cites column 17, lines 28-42 and 60-64, of Landers which describes a method for generating a reduced complexity genome (RCG) referred to as arbitrarily primed PCR (AP-PCR). The Office Action incorrectly equates the arbitrary primers of Landers et al. to the claimed degenerate primers. The arbitrary primers of Landers et al. are not degenerate. While the primers of Landers et al. have sequence that is arbitrarily chosen, the primers have a specific, non-random sequence. As Landers et al. notes, the primers used in AP-PCR are similar to the DOP-PCR primers with the exception that the AP-PCR primers consist only of the arbitrarily-selected nucleotides and not the 5' flanking degenerate residues or the tag present in primers for DOP-PCR (see column 17, lines 35-39). The arbitrarily-selected nucleotides of the AP-PCR primers are equivalent to the "TARGET" nucleotide sequence of the DOP-PCR primers. As Landers et al. notes (column 15, lines 18-20), the "'TARGET' nucleotide sequence includes at least 5 arbitrarily selected nucleotide residues that are the same for each primer of the set." The fact that the primers are specifically designed such that nucleotide residues in each of the primers of the primer set are the same excludes them from being degenerate. Thus, the primers of Landers et al. are not degenerate and thus are not the same as the claimed primers. Therefore, Landers et al. fails to disclose or suggest primers of degenerate sequence. Accordingly, Landers et al. cannot cure the deficiency in Lizardi.

The Office Action also cites, on page 8, lines 10-12, Figure 1 of Navarro et al as well as page 59, first paragraph; page 60, paragraphs 4 and 5 and page 61, first paragraph of Navarro et al. for allegedly teaching amplification of circular viriods using multiple random hexamers and AMV reverse transcriptase. The Office Action implies (and the rejection relies on the

assumption that) Navarro et al. discloses rolling circle amplification of a circular template using random primers as rolling circle replication primers. Navarro et al. does not disclose or suggest rolling circle amplification of a circular template and does not disclose or suggest amplification via random primers. The method of Navarro et al. involves reverse transcription of a circular RNA molecule via sequential first strand and second strand synthesis followed by PCR amplification of the resulting linear, double-stranded cDNA (see Section 2.2 bridging pages 60 and 61 and Figure 1). This is not rolling circle amplification and is does not use random primers for amplification. Rather, the method of Navarro et al. uses random primers merely to produce cDNA by reverse transcription which is then amplified via PCR of the linear, double-stranded cDNA. There is no nexus between the method of Navarro et al. and the methods of Lizardi and Landers et al. There is no reason why those of skill in the art would consider substituting the primers of Navarro et al. for the primers of Lizardi in the method of Lizardi and the Office Action points to no evidence of this. For at least these reasons, Navarro et al. does not correct the deficiencies in the disclosures of Lizardi and Landers et al.

Eckstein et al. which was cited for disclosing of modified nucleotides also fails to cure the deficiencies in Lizardi, Landers et al., and Navarro et al. discussed above.

The present rejection is impliedly based on the premise that it would be obvious to substitute any primer used in any replication reaction in any other replication reaction. This follows from the fact that the rejection fails to provide any meaningful nexus between the methods of the various prior art documents and fails to provide any specific motivation to make the required substitutions and modification to the prior art methods. This is not sufficient to establish obviousness. The fact that something could be combined with another thing is not enough to make such a combination obvious. There are numerous methods of replication and amplification of nucleic acids, and although the underlying process of such methods is similar (enzymatic addition of nucleotide monomers to a polynucleotide strand), such methods use a wide variety of primers, templates and relationships between primers and templates. Given this, it is not the case that primers can be generally substituted for any other primer. In such a case, it cannot be said that it would be obvious to substitute some certain primers in some certain

methods based on the premise that any primers in any methods of amplification can be substituted for one another. Yet, that is all that the present rejection provides, underneath it all, to provide a basis for the substitution of primers from disparate methods. For these additional reasons, the present rejection cannot be sustained.

For all of the above reasons, Lizardi, Landers et al., Navarro et al., and Eckstein et al., either alone or in combination, fail to make obvious claims 1, 5-9, 14, 20, 22-25, 27, 29, 35-38, 39, 41, 44-49, 51-53, 55-58, 69 and 70. As such, Applicant respectfully requests withdrawal of this rejection.

2. Claims 32, 42 and 59 were rejected under 35 U.S.C. § 103(a) as being unpatentable over Lizardi (U.S. Patent No. 5,854,033), Landers et al. (U.S. Patent No. 6,703,228), Navarro et al. (J. Virol. Meth., vol. 56, pp59-66, 1996) and Eckstein et al. (Trends in Bioch. Sci., vol. 14(3), pp. 97-100, 1989) and in further view of Skerra (Nucleic Acids Research, Vol. 20, pp. 3551-3554, 1992). Applicant respectfully traverses this rejection.

Claims 42 and 59 depend from claim 1 and therefore encompass all the limitations of claim 1. Applicant notes that the rejection applies Lizardi, Landers et al., Navarro et al., and Eckstein et al. in the same way and for the same disclosure for which Lizardi, Landers et al., and Eckstein et al. were applied in the rejection of claims 1, 5-9, 14, 20, 22-25, 27, 29, 31, 33, 35-38, 39, 41, 44-49, 51-53, 55-58, 69 and 70 under 35 U.S.C. § 103(a) discussed above. As discussed above, Lizardi, Landers et al., Navarro et al., and Eckstein et al., either alone or in combination, fail to disclose, suggest, or provide motivation for the use of primers of degenerate sequence in the method of Lizardi or hybridization of each amplification target circle to a plurality of primers such that multiple tandem sequence DNA products be produced by extension of the primers in rolling circle replication. Skeera et al. fails to supplement this gap in Lizardi, Landers et al., Navarro et al., and Eckstein et al.

Skerra et al. was cited for disclosure of incorporation of a phosphorothioate nucleotide at the 3'-end of the primer renders it inactive to the 3'->5' exonuclease activity of DNA polymerases such as Vent and Pfu and use of a mixture of exonuclease-sensitive and exonuclease-resistant primers in the amplification reaction. This does not supply what is missing

from Lizardi, Landers et al., Navarro et al., and Eckstein et al. Thus, Lizardi, Landers et al., Navarro et al., Eckstein et al., and Skeera et al., either alone or in combination, fail to disclose, suggest, or provide motivation for the use of primers of degenerate sequence in the method of Lizardi or hybridization of each amplification target circle to a plurality of primers such that multiple tandem sequence DNA products be produced by extension of the primers in rolling circle replication. Accordingly, Lizardi, Landers et al., Navarro et al., Eckstein et al., and Skeera et al. fail to make obvious claims 42 and 59. Applicant respectfully requests withdrawal of this rejection.

Pursuant to the above remarks, reconsideration and allowance of the pending application is believed to be warranted. The Examiner is invited and encouraged to directly contact the undersigned if such contact may enhance the efficient prosecution of this application to issue.

A Credit Card Payment Form PTO-2038 authorizing payment in the amount of $620.00, $395.00 for the fee for a small entity under 37 C.F.R. § 1.17(e) and $225.00 representing the fee for a small entity under 37 C.F.R. § 1.17(a)(2), a Request for Continued Examination and a Request For Extension of Time are enclosed. This amount is believed to be correct; however, the Commissioner is hereby authorized to charge any additional fees which may be required, or credit any overpayment to Deposit Account No. 14-0629.

Respectfully submitted,

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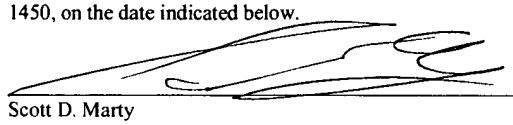

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12-7-06

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